Bovine Respiratory Disease: Sero-Epidemiological Surveys in Unvaccinated Cattle in Morocco

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ABSTRACT

Viral respiratory diseases are a major cause of severe clinical symptoms and substantial economic losses in cattle breeding. This study aimed to evaluate the epidemiological status of four respiratory viruses in cattle including Bovine Viral Diarrhea Virus/Mucosal Disease Virus (BVDV/MDV), Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis Virus (IBR/IPV), Bovine Respiratory Syncytial Virus (BRSV), and Bovine Parainfluenza Virus type 3 (PI3). A total of 1,741 blood samples were collected from 242 unvaccinated herds. The animals comprised 1314 females and 427 males, aged between 6 months and 5 years. The herds included both pure breeds (Holstein or Montbéliarde breeds) and crossbreeds (local-Holstein or local-Montbéliarde), and were located in 89 rural communes of six regions of Morocco including Casablanca-Settat, Rabat-Salé-Kénitra, Marrakech-Safi, Béni Mellal-Khénifra, Fès-Meknès, and Oriental. The samples were analyzed using the I-ELISA technique. The results indicated seroprevalence rates of 56.1, 21.5, 86.4, and 85.4% for BVD/MD, IBR/IPV, BRSV, and Parainfluenza-3, respectively. Co-infections were observed in 88% of the infected cattle, and 95% of the cattle were infected with at least one of the four viruses. Seroprevalence rates varied significantly with age, sex, breed, breeding systems, and practices. These findings confirmed the endemic status of bovine respiratory viral diseases and highlighted their direct and indirect impacts on livestock losses in Morocco.

Keywords: Bovine respiratory syncytial virus, Bovine viral diarrhea Virus/mucosal disease virus**,** Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus, Parainfluenza-3 Virus, Serology

INTRODUCTION

Bovine respiratory disease (BRD) is one of the most prevalent and economically significant diseases affecting both beef and dairy cattle worldwide (Fulton, 2009; Mehinagic et al., 2019). Among all possible causes of bovine respiratory disease, viruses are by far the primary etiological agents as they change the respiratory mucosa, produce cytokines, and impair the functioning of immune system cells (Bosch et al., 2013). Key viral contributors to this condition include bovine viral diarrhea virus/mucosal disease (BVDV/MD), infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus (IBRV/IPV), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus type 3 (PI3) (Fulton, 2009).

Bovine viral diarrhea virus (BVDV) is endemic in cattle populations worldwide. BVDV is a *pestivirus* within the family of *Flaviviridae*, related to the classical swine fever virus and the border disease virus (Vilcek et al., 2004). The economic impact of BVDV has grown with the emergence of more virulent strains since the 1980s and 1990s (Al-Kubat et al., 2021). Significant economic impacts associated with BVDV infection include reduced fertility, abortions, growth retardation, and the birth of calves that remain persistently infected with the virus. These calves are uniquely prone to contracting the lethal condition known as "mucosal disease" (Al-Mubarak et al., 2023).

Infectious bovine rhinotracheitis (IBR/IPV) is a contagious infectious disease caused by bovine herpesvirus 1 (BoHV-1), which belongs to the *Herpesviridae* family and the *varicellovirus* genus (Nandi et al., 2009). Its first discovery is traced back to the early 1950s in fattening units in the Western United States. In Europe, the first IBR/IPV outbreaks were reported in 1970 (Straub, 1975). IBR/IPV can cause respiratory, ocular, reproductive, central nervous system, enteric, neonatal, and cutaneous symptoms in cattle (Iscaro et al., 2021).

Bovine respiratory syncytial virus (BRSV) is one of the main viral infectious agents responsible for the onset of bovine respiratory disease (Sudaryatma et al., 2018). It was first identified as a cause of respiratory disease in the 1970s (Larsen, 2000). BRSV often results in primary infection of the respiratory tract and predisposes cattle to secondary

infections by bacterial pathogens (Tjønehøj et al., 2003; Agnes et al., 2013). This virus is a member of the *orthopneumovirus* genus within the family of *Pneumoviridae* (Rima et al., 2017). The disease resulting from infection with BRSV can range from subclinical causes to severe manifestations of clinical signs including nasal discharge, anorexia, coughing, pyrexia, and respiratory distress (Valarcher and Taylor, 2007).

The association of bovine parainfluenza virus 3 (PI3) with respiratory disease in cattle has been documented since its first discovery in 1959 (Gaudino et al., 2023). It is classified under the *respirovirus* genus within the *Paramyxoviridae* family (Albayrak et al., 2019). PI3 is now recognized as a widespread infectious agent in cattle populations around the world (Erid et al., 2024). The range of symptoms associated with PI3 infection can vary greatly, from mild or even unnoticed to severe respiratory illness, characterized by a runny nose, elevated body temperature, and persistent coughing (Newcomer et al., 2017). Moreover, cattle infected with PI3 often experience concurrent viral or bacterial infections, suggesting that PI3 may compromise the immune system and facilitate these subsequent infections (Newcomer et al., 2017).

In Morocco, few studies have been conducted on bovine respiratory diseases. An early study on 524 cattle from different areas of the country reported seroprevalences of 48.5% for BVDV, 62.8% for Bovine Herpesvirus Type 1, 70.4% for BRSV, and 68.1% for PI3 (Mahin et al., 1985). Alali et al. (1992) conducted a clinical study on BRD in the Gharb region in Morocco and reported morbidity, mortality, and lethality rates of 30.6%, 1.6%, and 5.1%, respectively. Lucchese et al. (2016) identified the primary causes of abortion in dairy cattle in Morocco and reported seroprevalence rates of up to 37.71% for BVDV and 50% for BHV-1. The findings suggest that bovine respiratory viruses are endemic and may have circulated at high levels in the country for a long time.

In 1975, the Moroccan government launched the 'Dairy Plan' to intensify dairy farming and develop milk production, particularly in large urban areas and irrigated coastal regions (Chatibi, 2011). In 2008, the 'Green Morocco Plan' was introduced to bridge the gaps between small, medium, and large farms by modernizing and aggregating them into competitive agro-industrial chains (FAO, 2011). These government initiatives transformed the structure of livestock farming in Morocco, not only enhancing production and productivity but also increasing the prevalence and severity of diseases associated with the industrialization and intensification of livestock farming, particularly respiratory infections (Moroccan Ministry of Agriculture, 2015). Respiratory diseases often occur discreetly and without indicative clinical signs (Patrick and Gorden, 2010). However, under stressful conditions, particularly when transporting and assembling animals at livestock markets, one or more of these diseases may show clinical manifestation, occasionally with very severe symptoms leading to animals' deaths (Patrick and Gorden, 2010). Significant indirect losses such as decreased milk and meat production and increased medical services are often associated with these diseases (ONSSA, 2018). Vaccines against respiratory viruses are available in the Moroccan pharmaceutical market, but only IBR/IPV is a notifiable disease under Moroccan legislation, requiring adherence to a code of procedure established by national authorities (ONSSA, 2018). However, vaccination alone is insufficient to control BVD/MD, IBR/IPV, BRSV, and PI3 if their risk factor management and biosecurity measures are not properly implemented (Makoschey and Berge, 2021). In this regard, the present study aimed to assess the epidemiological status of BVD/MD, IBR/IPV, BRSV, and PI3 in cattle throughout Morocco. It also sought to evaluate the impact of various risk factors associated with these diseases, such as age, sex, breed, mode of reproduction, rearing method, rearing conditions, interaction and movement between farms, usable agricultural area, herd size, and the origin of the cattle on their transmission.

MATERIALS AND METHODS

Ethical approval

All animals were handled according to international laws concerning animal welfare and handling. Blood samples were collected by a veterinarian in a non-stressful environment. Breeders and owners were informed of the objectives and nature of the analysis.

Study period and regions

This cross-sectional study encompassed 242 farms across 89 rural communities in six regions of Morocco (out of a total of 12 regions) known for their high concentration of livestock production, namely Casablanca-Settat, Rabat-Salé-Kénitra, Marrakech-Safi, Béni Mellal-Khénifra, Fès-Meknès, and Oriental. The study was conducted during the cold season from December 2018 to March 2019. A total of 1,741 blood samples were collected (Figure 1).

Selection of farms and animals

Farms were selected to cover as many rural communities as possible and to represent various types of farming and cattle categories. No clinical correlation was sought in this study, which was designed to determine seroprevalence without regard to the clinical aspects of the viral infections under study. Farm sizes ranged from small herds of 10 animals to large herds of 170. In each farm, a random sample comprising approximately 30% of the total number of cattle in each farm was chosen. As illustrated in Figure 2, the sampling plan within each holding was designed to reflect the structure of cattle farming at the national level.

Furthermore, since there is no way (under this study conditions) to differentiate between vaccinated animals and animals that had/have the diseases, they were excluded from the present study: first, calves younger than 6 months of age (to avoid potential serological interference with the colostral antibodies); second, cattle that are from a region in Morocco (Souss-Massa region) where most animals are usually vaccinated against the four diseases; third, cattle from vaccinated herds within the study regions; and fourth, imported calves from endemic countries, where they typically receive vaccination against these viruses followed by a booster once they reach Morocco.
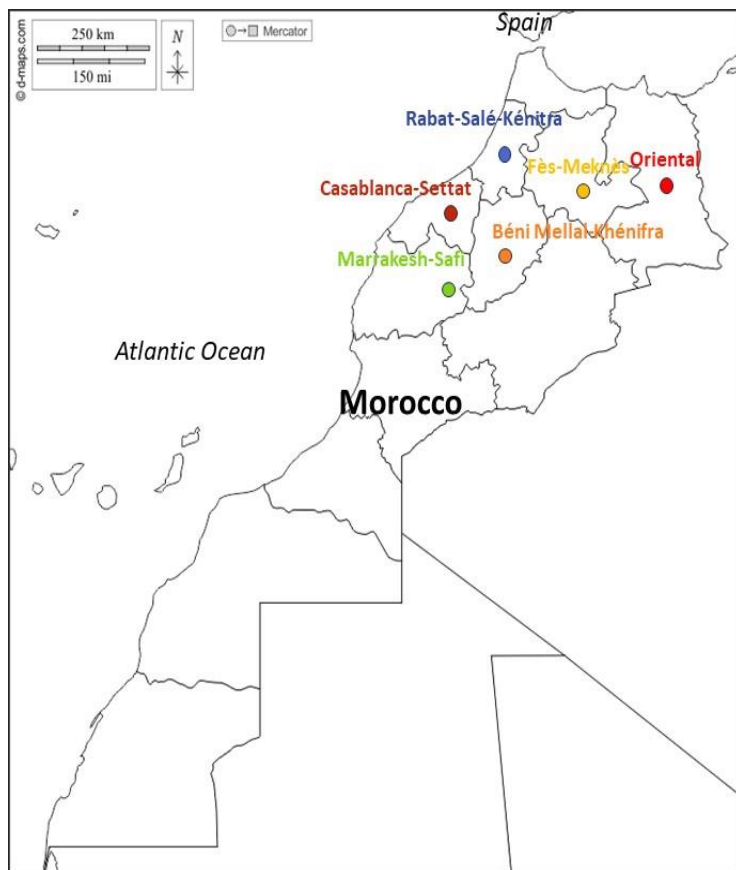

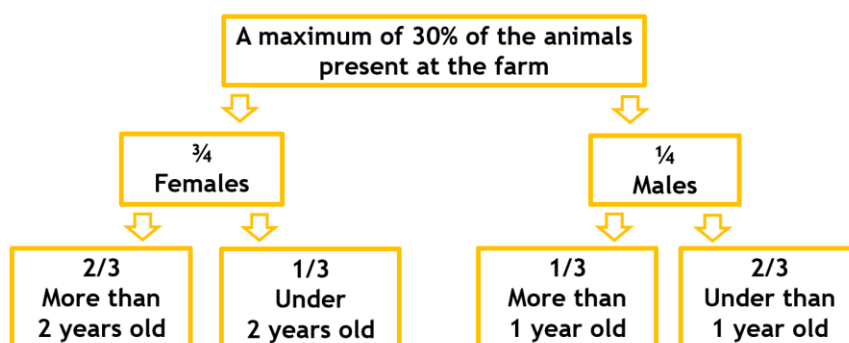**Figure 1.** The different regions of the study area in Morocco during 2018-2019

Figure 2. Survey methodology across the sampled farms in the different regions of the study area in Morocco during 2018-2019

Samples

Blood samples were collected from the jugular or coccygeal vein using 10 ml sterile dry tubes and transported to the laboratory at +4°C. After centrifugation (15 minutes at 3000 rpm) and serum collection, the samples were stored at -20°C. For each tested animal, an epidemiological survey was performed to determine (1) the age of the animal (> 6 months and < 1 year, 1 to 2 years, > 2 years), (2) sex, (3) housing conditions (good, average, poor), (4) contact with animals from other farms (yes, No), (5) herd size (1 to 10 cattle, 11 to 40 cattle, more than 40 cattle), (6) reproduction methods (artificial insemination, natural mating, mixed), (7) breeds (crossbred (local-Holstein or local-Montbéliarde), pure breeds (Holstein or Montbéliarde), (8) usable agricultural area (UAA; < 5 ha, 5 to 10 ha, > 10 ha), (9) origin of the animals (imported, born and raised in Morocco), (10) farming methods (extensive, semi-intensive, intensive, fattening, mixed [extensive- intensive- fattening]), and 11) the region under study.

Serological tests

The collected sera were analyzed utilizing indirect enzyme-linked immunosorbent assay (I-ELISA). The IBR-Ab, BVDV-Ab, BRSV-Ab, and PIV3-Ab kits from SvanovaBiotech AB (Uppsala, Sweden) were used according to the manufacturer's instructions to detect specific IgG antibodies against IBR/IPV, BVDV, BRSV, and PIV3, respectively.

The results obtained with the I-ELISA kits used required calculating the optical corrected densities (ODcorr) by subtracting the OD values of the wells containing the control antigen (ODctrl) from the corresponding viral antigen-coated wells (ODatg), i.e. (ODcorr=ODatg- ODctrl). Percentage positivity (PP) was then calculated as follows:

$$PP = (OD_{corr} \text{ of test sample}) \times 100/ (OD_{corr} \text{ of positive control})$$

The interpretation of the PP into a seropositive/seronegative infectious status was done using the tables provided by the manufacturer.

Statistical analysis

The chi-square independence (chi-square distribution) test was used to assess the possible association between the seroprevalence rates and the different risk factors considered. The study considered a p-value of less than 0.05 as the threshold for statistical significance. This test was conducted using the multiple correspondence factor analysis (MCFA) method. All statistical tests were performed using SPSS software (Modeler 16.0).

RESULTS

Seroprevalence overall and by study area

The overall infection seroprevalence rates found were 56.1% for BVD, 21.5% for IBR/IPV, 86.4% for BRSV, and 85.4% for PI3. The variation of seroprevalence by region is presented in Table 1.

Transmission risk factors of the four studied diseases

Seroprevalence rates for the four studied diseases varied significantly by age, sex, breed of cattle, and breeding systems and practices. The seroprevalence rates according to the different epidemiological factors are presented in Table 2.

a. Bovine viral diarrhea/mucosal disease

The results of the analysis showed a significant association ($p < 0.05$) between BVD/MD seroprevalence and the breeding methods. The fattening farms had the highest rate (73.50%), while extensive farms had the lowest rate (33.33%). In terms of age and sex, older animals (67.38%) and females (59.97%) had the highest rates compared to younger animals (39.72%) and males (44.03%, $p < 0.05$). The breed of animals also significantly impacted BVD/MD seroprevalence, with pure breeds (62.05%) more frequently infected than crossbreds (54.33%, $p < 0.05$).

However, no statistically significant association was observed between the seroprevalence of BVD/MD and herd size, reproductive methods (artificial insemination, natural mating, mixed), housing conditions, and whether there was contact or not with animals from other farms ($p > 0.05$). The multiple correspondence factor analysis (MCFA) identified two factors of the first and second dimensions with an explained percentage variation of 44.6%, which is considered quite satisfactory. In other words, this method explains 44.6% of the total variation of the cloud of points representing the risk factors likely to influence the observed BVD/MD seroprevalence. The projection of the studied parameter modalities on axis 1 (explaining 25.67% of this variation) and axis 2 (explaining 18.89% of the same variation, Figure 3) showed a correspondence between the practice of artificial insemination, animals over 2 years old, females and the high seroprevalence of BVD/MD in the Rabat-Salé-Kénitra and Casablanca-Settat regions. In these two regions, the farms most affected by BVD/MD were those that practiced artificial insemination on adult females (over two years old). In contrast, cattle purchased locally in the Fès-Meknès region were moderately infected with BVD/MD, while male cattle under one year of age from the eastern region were the least affected.

Table 1. Seroprevalence of bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, and bovine parainfluenza virus according to the different studied regions in 2018-2019.

Region	Number of samples	BVD seroprevalence (%)	IBR/IPV seroprevalence (%)	BRSVs seroprevalence (%)	PI3 seroprevalence (%)
Casablanca-Settat	446	59.42	24.77	88.79	89.91
Béni Mellal-Khénifra	283	48.76	15.41	88.34	87.28
Rabat-Salé-Kénitra	365	59.73	20	83.01	82.19
Fès-Meknès	78	60.26	25	78.2	82.05
Oriental	221	50.23	22.83	81.9	80.09
Marrakech-Safi	348	56.61	22.09	89.94	85.34
Total	1741				
Overall seroprevalences		56.06	21.48	86.39	85.35

BVD: Bovine Viral Diarrhea virus, IBR/IPV: Infectious bovine rhinotracheitis virus/Infectious Pustular Vulvovaginitis Virus, BRSV: Bovine respiratory syncytial virus and PI3: Bovine para-influenza virus

Table 2. Seroprevalence of BVD, IBR/IPV, BRSV, and PI3 according to the categories of epidemiological studied factors in the different regions of the study area in Morocco during 2018-2019.

Breeding parameters	Categories	Number of sera tested	Seroprevalences (%)			
			BVD*	IBR/IPV*	BRSV*	PI3*
UAA	< 5 ha	1080	56.85	18.94	86.02	85.09
	5 to 10 ha	351	50.43	26.72	86.61	88.03
	> 10 ha	310	59.68	24.34	87.42	83.23
			$(p > 0.05)$	$(p > 0.05)$	$(p > 0.05)$	$(p > 0.05)$
Origin of the animals	Imported	347	55.33	22.51	86.17	83.57
	Born and raised in Morocco	1237	57.72	21.16	86.18	84.56
	Mixed origin	157	44.59	21.66	88.53	95.54
			$(p < 0.05)$	$(p > 0.05)$	$(p > 0.05)$	$(p < 0.05)$
Breeding methods	Extensive	12	33.33	36.36	91.67	83.33
	Semi-Intensive Dairy	872	58.14	16.76	86.47	83.72
	Dairy Intensive	384	55.47	24.01	89.84	89.58
	Fattening	83	73.50	35.8	89.16	93.98
	Mixed	388	49.22	26.31	82.22	82.99
			$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$
Herd size	1 to 10 cattle	292	57.88	17.3	81.16	78.08
	11 to 40 cattle	1073	55.36	17.64	87.5	86.58
	More than 40 cattle	376	56.65	35.77	88.56	87.5
			$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$
Mode of reproduction	AI	1191	56.09	21.14	86.98	87.49
	Natural mating	279	57.35	16.55	85.3	77.78
	Mixed	201	47.76	32.49	84.58	82.59
			$(p > 0.05)$	$(p < 0.05)$	$(p > 0.05)$	$(p < 0.05)$
Breed	Crossbreed	1351	54.33	20.23	86.38	85.05
	Pure breed	390	62.05	25.78	86.41	86.41
			$(p < 0.05)$	$(p > 0.05)$	$(p > 0.05)$	$(p > 0.05)$
Age	> 6 months and < 1 year	287	39.72	7.34	64.46	63.07
	1 to 2 years	568	46.65	14.59	80.28	79.05
	>2 years	886	67.38	30.57	97.4	96.61
			$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$
Sex	Female	1314	59.97	24.73	91.7	90.49
	Male	427	44.03	11.56	70.02	69.55
			$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$	$(p < 0.05)$
Breeding conditions	Good	143	57.34	32.12	86.71	83.92
	Average	1196	55.6	21.49	86.7	85.03
	Bad	393	58.26	18.2	85.75	86.77
			$(p > 0.05)$	$(p < 0.05)$	$(p > 0.05)$	$(p > 0.05)$
Contact with animals from other farms	Yes	55	56.36	3.7	92.73	92.73
	No	1169	54.58	20.14	86.48	85.46
			$(p > 0.05)$	$(p < 0.05)$	$(p > 0.05)$	$(p > 0.05)$

*BVD: Bovine Viral Diarrhea virus, IBR/IPV: Infectious bovine rhinotracheitis virus/infectious pustular vulvovaginitis Virus, BRSV: Bovine respiratory syncytial virus and PI3: Bovine para-influenza type 3. UAA: Usable agricultural area; ha: Hectare.

a. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

The IBR/IPV seroprevalences observed in this study were significantly influenced by farm size ($p < 0.05$). Larger farms exhibited higher seroprevalence rates compared to smaller farms (35.77% versus 17.30%). The type and conditions of husbandry also significantly affected seroprevalence ($p < 0.05$): paradoxically, the virus was more prevalent in optimal husbandry conditions than in average or poor conditions (32.12%, 21.49%, and 18.20%, respectively). Additionally, the sex and age of the animals had a significant impact ($p < 0.05$) with females (24.73%) and older animals (30.57%) showing higher rates than males (11.56%) and younger animals (7.34%). However, the origin and breed of the animals did not significantly affect the seroprevalence of IBR/IPV in this study ($p > 0.05$).

In MCFA, all variables are loaded onto two factors representing the first and second dimensions, explaining 44.5% of the variance, which is considered satisfactory. These two dimensions effectively summarize 44.5% of the information provided by the parameters for IBR/IPV, reflecting an important relationship between IBR/IPV seroprevalence and the studied parameters. From the projection of the breeding parameter modalities onto the two axes (Figure 4), it can be concluded that farms in the Marrakech-Safi region, which were less affected by the IBR/IPV virus, practice natural mating and have more male cattle under one year of age. Farms significantly affected by IBR/IPV in the Rabat-Salé-Kénitra region had average breeding conditions, practiced artificial insemination, and kept females over two years old ($p < 0.05$).

b. Bovine respiratory syncytial virus infection

The analysis of the seroprevalence of BRSV according to different epidemiological parameters showed a significant impact of sex and age on the seroprevalence rates ($p < 0.05$). Females and older animals had higher rates compared to males and younger ones (91.70% and 97.40% versus 70.02% and 64.46%, respectively). Likewise, herd size significantly impacted BRSV infection ($p < 0.05$), with larger farms being more frequently affected than smaller farms (88.56% versus 81.16%, respectively). However, no significant dependence was observed between BRSV seroprevalences and UAA, the origin of the animals, mode of reproduction, breed of the animals, housing conditions, or contact between cattle from different farms ($p > 0.05$). The analysis conducted using MCFA revealed two primary factors: the first accounts for 25.69% of the variability, while the second accounts for 19.42%. Together, they explain 45.1% of the total variance, which is deemed to be quite satisfactory. The data visualization indicates that in the Casablanca-Settat region, the Bovine Respiratory Syncytial Virus (BRSV) significantly impacted farms with female cattle older than two years (Figure 5). In contrast, farms in the Rabat-Salé-Kénitra region, which were moderately affected, maintained average hygiene standards and used artificial insemination. Meanwhile, in the Fès-Meknès region, farms that were minimally affected by the virus tended to use natural breeding methods and had a higher presence of crossbred cattle. Notably, the lowest rates of seroprevalence were observed in male cattle aged between six months and one year.

c. Infection with bovine parainfluenza virus 3

The statistical analysis demonstrated a strongly significant association between the seroprevalence of PI3 and several factors, including herd size, reproductive methods, age, and sex of the animals ($p < 0.05$). Larger herds (87.57%) were more affected by PIV-3 compared to smaller herds (78.08%). Farms practicing artificial insemination showed a higher seroprevalence (87.49%) compared to those practicing natural mating. Similarly, cattle over two years of age (96.61%) and females (90.49%) had higher seroprevalence rates compared to those under one year of age (79.05%) and males (69.55%). However, no statistically significant association was observed between PI3 seroprevalence and UAA, animal breed, husbandry conditions, and contact between animals from different farms ($p > 0.05$). The evaluation of all variables revealed two primary factors on the first dimension, accounting for 25.7% of the variance, and two factors on the second dimension, explaining 19.4% of the variance. Together, these factors contributed to a total explained variance of 45.1%, which is considered quite satisfactory. The analysis of the modalities across two dimensions (Figure 6) indicates that farms performing artificial insemination (AI) and those with a greater population of females over two years old had higher seroprevalence rates of PI3. This trend was particularly noticeable in Casablanca-Settat and Rabat-Salé-Kénitra regions. In the Fès-Meknes region, the most affected farms were those with locally purchased crossbred animals in substandard hygienic conditions. The lowest levels of seropositivity were found among the youngest male animals, particularly those aged between six months and one year.

d. Co-infections

In this study, only 12% of the tested animals were seropositive for one single virus. Co-infections were frequently observed (88%) across all the regions under study. The most frequent associations were BRSV-PIV-BVD (34.06%), followed by BRSV-PIV (23.78%), and BRSV-PIV-BVD-IBR/IPV (15.33%, Figure 7). Furthermore, it should be noted that only 5% of the studied animals were not seropositive for any of these four infections. This finding highlights the large distribution of these diseases across the studied regions.
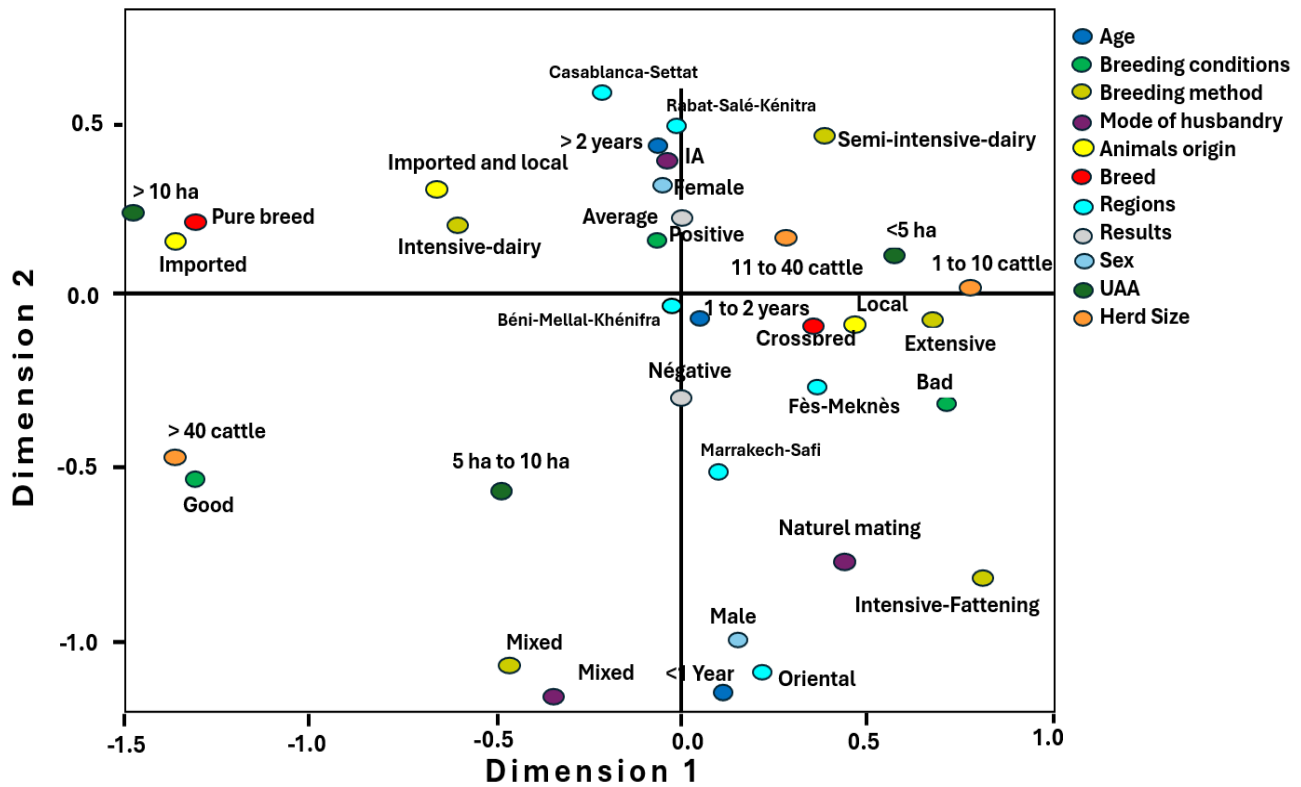
Figure 3. Different parameter modalities concerning bovine viral diarrhea/mucosal disease infection in cattle of Morocco during 2018-2019. The projection of the studied parameter modalities on axis 1 (explaining 25.67% of the variation) and axis 2 (explaining 18.89% of the variation) revealed a correspondence between the practice of artificial insemination, animals over 2 years old, females, and Rabat-Salé-Kénitra region.
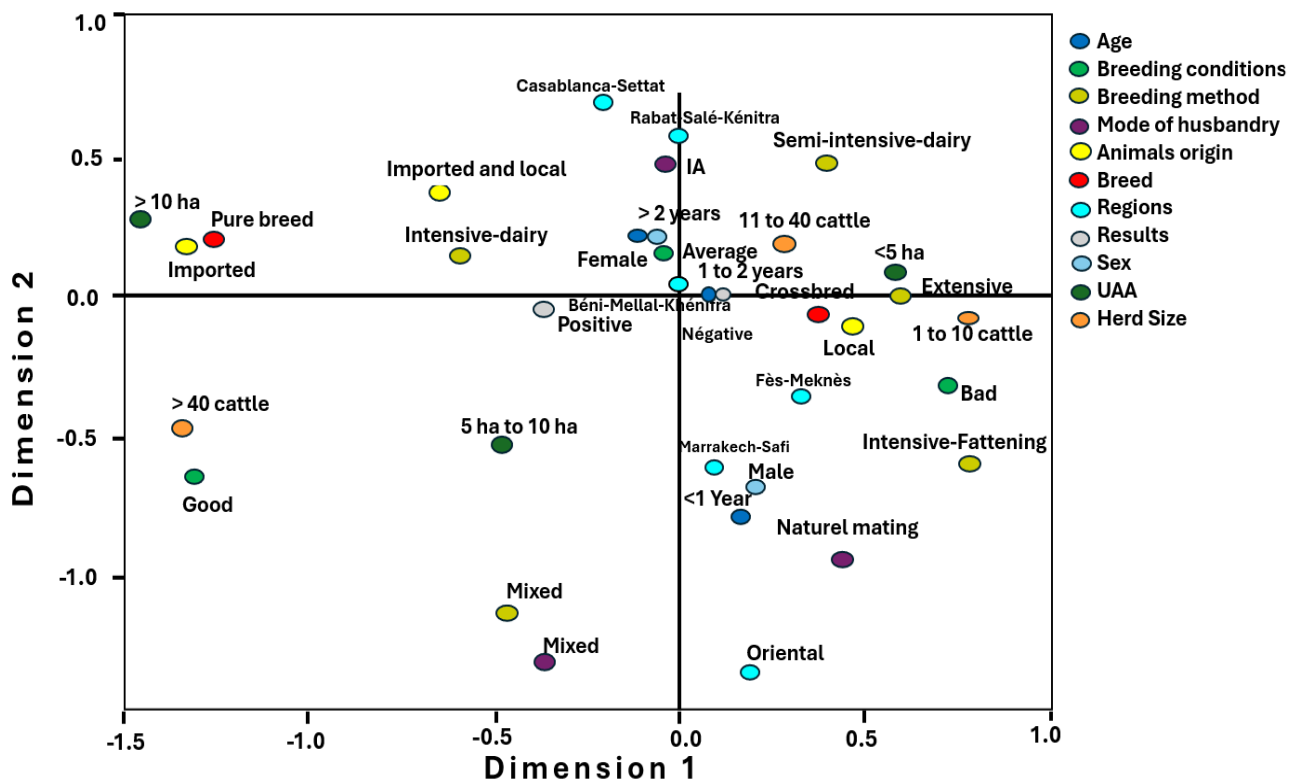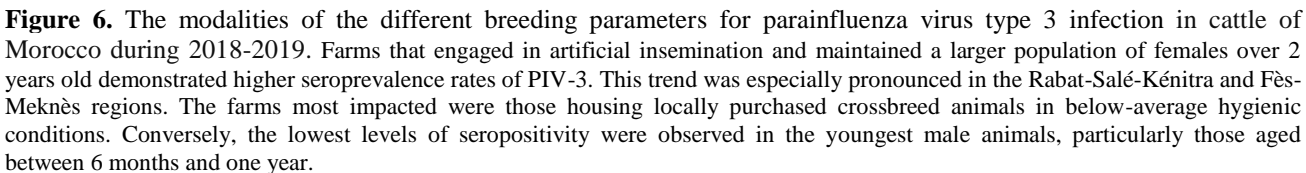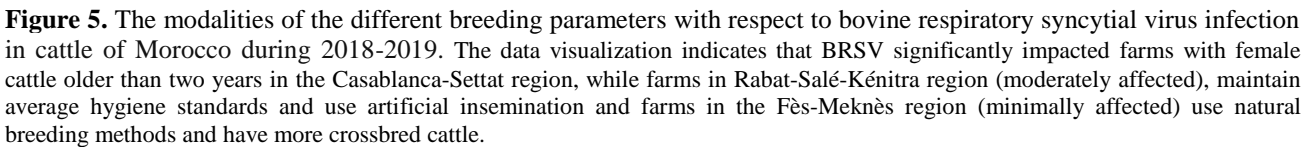

Figure 4. The modalities of the different parameters for bovine rhinotracheitis infection in cattle of Morocco during 2018-2019. The breeding parameter modalities onto the two axes: The Marrakech-Safi region that was weakly affected by the IBR/IPV virus practices natural mating and has more male cattle under one year of age. Farms significantly affected by IBR/IPV in the Rabat-Salé-Kénitra region had average breeding conditions, practiced artificial insemination, and kept females over 2 years old.
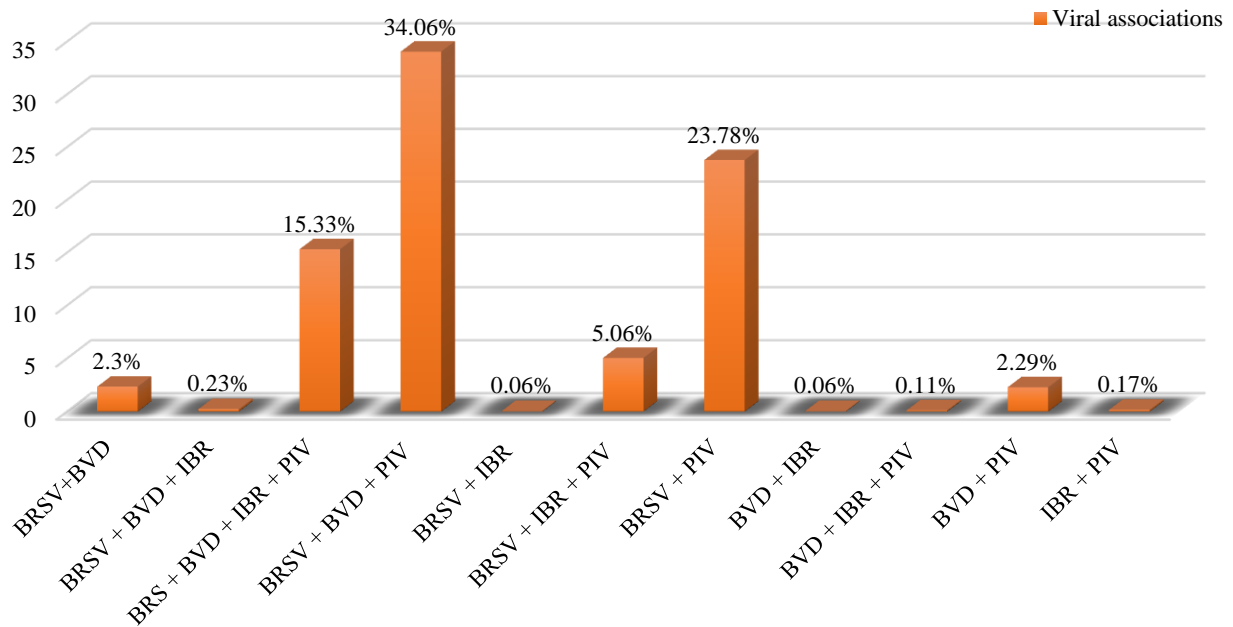
Figure 5. The modalities of the different breeding parameters with respect to bovine respiratory syncytial virus infection in cattle of Morocco during 2018-2019. The data visualization indicates that BRSV significantly impacted farms with female cattle older than two years in the Casablanca-Settat region, while farms in Rabat-Salé-Kénitra region (moderately affected), maintain average hygiene standards and use artificial insemination and farms in the Fès-Meknès region (minimally affected) use natural breeding methods and have more crossbred cattle.

Figure 6. The modalities of the different breeding parameters for parainfluenza virus type 3 infection in cattle of Morocco during 2018-2019. Farms that engaged in artificial insemination and maintained a larger population of females over 2 years old demonstrated higher seroprevalence rates of PIV-3. This trend was especially pronounced in the Rabat-Salé-Kénitra and Fès-Meknès regions. The farms most impacted were those housing locally purchased crossbreed animals in below-average hygienic conditions. Conversely, the lowest levels of seropositivity were observed in the youngest male animals, particularly those aged between 6 months and one year.

Figure 7. The distribution of various co-infection combinations by the four studied viruses in cattle of Morocco during 2018-2019. The occurrence of co-infection was frequently observed (88%). The most frequent associations were BRSV-PIV-BVD (34.06%), followed by BRSV-PIV (23.78%, and BRSV-PIV-BVD-IBR/IPV).

DISCUSSION

The results of this study indicated that the four viruses involved in bovine respiratory syndrome, namely BVD, IBR/IPV, BRSV, and PIV-3, are circulating in the studied regions with relatively high seroprevalence rates of 56.1%, 21.5%, 86.4%, and 85.4%, respectively. Additionally, 95% of the animals were infected with at least one of these viruses. This underscores the impact of both direct and indirect losses associated with these diseases on livestock in Morocco. However, there have been few comprehensive studies at the national level to assess the epidemiological status, transmission routes, and extent of these diseases, which are critical for managing and controlling their spread with worldwide effects (Gaudino et al., 2022).

Seroprevalence rates of the four diseases

BVDV is a cosmopolitan infection that has been reported on all continents, with seroprevalences ranging from 20% to 90% (Mahmoud and Allam, 2013). Similar seroprevalence rates to those observed in the present study were reported in Brazil (57.7%, Frandoloso et al., 2008), and Switzerland (56%, Schweizer et al., 2011). Higher seroprevalence rates were reported in many other countries such as Thailand (73%, Kampaa et al., 2004), Ethiopia (80 %, Tesfaye et al., 2021), Mexico (87.8%, Gael et al., 2016), and Canada (90%, Ahmad et al., 2011). However, lower seroprevalence rates were reported in Egypt (31.5%, Maher et al., 2023), Saudi Arabia (26%, Mahmoud and Allam, 2013), Belgium (32.9%, Sarrazin et al., 2013), and Jordan (31.6%, Talafha et al., 2009). Moreover, the rate observed in this study was higher than those reported in similar previous studies conducted in Morocco, particularly those conducted by Aiyar (2006) and Mahin et al. (1985) with respective rates of 39.53% and 48.5%. This suggests an increasing spread of the BVD/MD virus at the national level. The observed seroprevalence of bovine herpesvirus type 1 in this study (21.48%) is lower than those reported in several countries, such as Mexico (89.85%, Cordova et al., 2009), Uruguay (37%, Guarino et al., 2008), Thailand (67%, Kampaa et al., 2004), China (35.8%, Yan et al., 2008), and Egypt (75.80%, Amal et al., 2008). However, this rate is similar to those observed in Mexico (85.6%, Calderon et al., 2007) and Turkey (88.82%, Okur et al., 2007), yet it is significantly higher than the rate reported in Saudi Arabia (69.1%, Mahmoud and Allam, 2013). For BRSV, the seroprevalence rate observed in this study (86.39%) was higher than that found previously in Morocco by Aiyar (2006, 78.68%) and Mahin et al. (1985, 70.40%). However, it is similar to those reported in Mexico (85.5%) and Venezuela (85%; Calderon et al., 2007) and higher than those observed in the United States (56.6%; Grubbs et al., 2001) and Saudi Arabia (75.5%; Mahmoud and Allam, 2013).

The seroprevalence rate of PIV-3 infection observed in this study (85.35%) is higher than the rates recorded by previous studies in Morocco, namely those conducted by Aiyar (2006) (67.93%) and Mahin et al. (1985) (68.1%), which suggests an increase in the circulation of this infection during the last years among cattle in Morocco. Nonetheless, this rate is similar to those observed in Mexico (85.6%; Calderon et al., 2007) and Turkey (88.82%; Okur et al., 2007) although it is significantly higher than the rate reported in Saudi Arabia (69.1%; Mahmoud and Allam, 2013).

Evaluation of the impact of different epidemiological factors

The analysis of the seroprevalence variation of the four studied diseases according to different epidemiological parameters revealed that the most significant and common influence on these rates was due to the age and sex of the tested animals. Indeed, for the studied infections, seroprevalence rates increased significantly with age, and females were more frequently seropositive than males. This finding indicated that the risk of animals being infected by the viruses increases over time and suggests that the animals are regularly infected. Furthermore, the observed greater incidence of infections in females relative to males may be attributed to the fact that females are typically older than males at the time of slaughter. This is because female cattle are often kept for extended durations for reproductive purposes, allowing multiple births before they are slaughtered. In this study, more than 75% (1,314/1,741) of the studied animals were females; male cattle are fattened and sold when they are still young. Seemingly, then, the age of animals influences the variation of the seroprevalence rates of the four diseases more than their sex. This finding is supported by many other studies that have reported that the risk of infection by the four viruses increased with the age of animals (Calderon et al., 2007; Nandi et al., 2009; Talafha et al., 2009). Previous studies, including those of Autorino et al. (2002) and Houoiten et al. (2021), indicated that a significant increase in seroprevalence rates for a specific disease or infection correlating with the age of animals in a given area suggests consistent exposure to the pathogen over the years. Essentially, this means that in areas where a disease is endemic, it is common for animals to be regularly infected. This observation, coupled with the high seroprevalence rates identified in the present study, serves as evidence that the four diseases are indeed widespread and established within the country.

Animal breeds have also shown a significant impact on seroprevalence rates. Indeed, the seroprevalence rates, as mentioned before, were higher among the imported pure breeds (Holstein, Montbeliarde) than the local crossbreds (local-Holstein and local-Montbeliarde). According to previous studies, the breed of the animals (Snowder et al., 2006; Hussain et al., 2019) significantly influences the susceptibility or resistance of cattle to respiratory infections. Indeed, breeds such as Holstein, Charolais, Jersey, Friesian, and Friesian-Sahiwal seem to be more susceptible to the Bovine Respiratory Syndrome than the native local ones (Aiyar, 2006; Schelcher, 2008; Daves et al., 2016).

The present study also revealed that other factors, such as breeding methods and conditions, mode of reproduction, and the size of the herd, can also influence the seroprevalence rates of the four studied diseases. As mentioned earlier, several governmental programs have been launched in Morocco to intensify dairy farming and develop milk production. The industrialization and intensification of farming practices come with several factors favoring the transmission and spread of respiratory diseases, namely overcrowding, stress (from transport, feeding, etc.), confinement of animals in unfavorable hygienic and aeration conditions, etc. Moreover, many other studies have observed that the seroprevalence of respiratory infections is influenced by the employed breeding strategies and management practices. The impact of breeding systems and practices on respiratory disease infection rates was also observed in several other studies (Calderon et al., 2007; Schelcher, 2008; Cordova Izquierdo et al., 2009). Likewise, the present study revealed a significant association between artificial insemination practice, females aged more than two years, and the high infection rates of the studied respiratory diseases. The production and use of semen in cattle are consistently monitored for sexually transmitted diseases, which minimizes the likelihood of these infections spreading via semen. However, the observed results in the current study may be due to the use of artificial insemination primarily in adult female cattle that are kept for breeding purposes, and, as previously mentioned, they have a higher susceptibility to infection over time. Furthermore, the findings indicated a high prevalence of co-infections among the tested animals, with 88% showing seropositivity for multiple viruses. Co-infections can modulate disease outcomes, potentially exacerbating the severity of diseases. For instance, the presence of one pathogen can impair the host's immune response, making it more susceptible to other infections, or conversely, prompt an immune response that inhibits the replication of another pathogen. In terms of transmission dynamics, co-infections can influence the rate and method of disease spread. The interaction between different pathogens may lead to synergistic effects that enhance transmission or competitive interactions that reduce it (Kumar et al., 2018; Devi et al., 2021). Consequently, understanding the specific interactions between co-infecting pathogens is crucial for developing effective control measures.

CONCLUSION

This study has established that the four viruses involved in bovine respiratory syndrome are widespread in Morocco, with current seroprevalence rates significantly surpassing previous records. Consequently, it is imperative to manage and mitigate the transmission of these diseases, given the substantial direct and indirect losses they inflict on Moroccan livestock. While vaccination is strongly advised, it becomes futile without the concurrent implementation of biosecurity protocols, which include optimized architectural design for animal housing, improvement of hygiene conditions, reduction of stress and confinement, regular cleaning and disinfection of instruments and equipment used, AI control, elimination or isolation of infected animals, and animal movement control. However, the application of these control strategies is not without its challenges and limitations. Factors such as limited resources, infrastructural deficits, and cultural practices should be acknowledged. Indeed, these factors can significantly influence the practicality and success of the control measures. On the other hand, understanding the genetic characterization of these viruses may lead to more targeted and effective prevention strategies, ultimately contributing to better management of bovine respiratory diseases. Indeed, further studies focusing on the genomic characterization of the circulating viruses for medical prevention purposes are necessary at the national level.

DECLARATIONS

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding and the first author on reasonable request.

Competing interests
The authors declare no conflict of interest.

Authors' contributions
Said Alali made a major contribution to the study design, realization, and supervision of laboratory work, data analysis and interpretation, and redaction of the manuscript. Ikhlass El Berbri made a major contribution to study design, data analysis, and interpretation, redaction of the manuscript, and preparation of the final version of the manuscript. Fatima Zahra Laabouri contributed to the study design, data analysis and interpretation, and redaction of the manuscript. Imane Choukri revised the final version of the manuscript, submission of the manuscript, and coordinated the authors. Outenrhrine Hassan coordinated between commune chiefs and farm managers, conducted fieldwork, collected blood samples, and participated in data acquisition. El Ghourdaf Abdelmouni coordinated between commune chiefs and farm managers, conducted fieldwork, collected blood samples, and participated in data acquisition. All authors have read and given final approval for the last edition of the article to be published.

Ethical considerations
All authors were screened for ethical issues, including plagiarism, consent for publication, misconduct, fabrication of data, and duplicate publication or submission.

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